

"Deficiency of the Planar Cell Polarity protein CELSR3 in CF bronchial epithelial cells increases susceptibility to epithelial–mesenchymal transition"

Noël, Sabrina ; Dhooghe, Barbara ; Dykmans , Arnaud ; Delbart, Wendy ; Leal, Teresinha

Abstract

Objectives: Epithelial barrier function is impaired in CF airways as an indirect consequence of F508del-CFTR trafficking defect. The role of apico-basal polarity is crucial in the process but little is known about the influence of Planar Cell Polarity (PCP) which coordinates alignment of cell polarity across the tissue plane. Methods: Expression of several PCP proteins including atypical cadherin CELSR3 (Cadherin EGF LAG seven-pass G-type receptor 3) were quantified in normal and CF primary human bronchial epithelial (HBE) cells and HBE cell lines. To characterize the role of CELSR3 in epithelial polarity, CELSR3, epithelial and mesenchymal markers expression were quantified after inducing epithelial–mesenchymal transition (EMT) in HBEs monolayers with TGF β or cisplatin. To determine if CELSR3, suspected to undergo homophilic interactions to establish cell–cell interactions, is a key determinant of epithelial barrier function, transepithelial electric resistance (TEER) was measu...

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32 Mediation of pro/anti-inflammatory balance by vardenafil in mouse CF macrophages is dependent on CFTR expression

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Objectives: Having recently demonstrated that vardenafil, a phosphodiesterase type 5 inhibitor, reduces LPS-induced inflammatory responses in CF mice, we hypothesized that CF macrophages are characterized by a pro-inflammatory phenotype which can be modulated by vardenafil. We also want to study the role of CFTR in this vardenafil-induced immunomodulation of macrophages.

Methods: Macrophages were purified from lung homogenates from homozygous F508del-CFTR, CFTR^{-/-} (KO) and wild-type (WT) mice. To test the hypothesis that macrophages activity is altered in F508del-CF and KO mice, macrophages differentiation in pro-inflammatory (M1) effectors was studied after polarization with LPS and IFN- γ . Pro-inflammatory mediators TNF- α and NOS-2 were quantified by ELISA or by quantitative RT-PCR.

Results: F508del-CF lung macrophages displayed an exaggerated pro-inflammatory response to M1 mediators. Both TNF- α and NOS-2 levels were more than doubled. Similar observations were made in macrophages isolated from KO mice, confirming that loss of CFTR promotes pro-inflammatory phenotype in macrophages. In F508del-CF mice, vardenafil reduced the expression of pro-inflammatory mediators by at least 50%. However, vardenafil failed to normalize TNF- α and NOS-2 expression in KO macrophages, suggesting that the presence of CFTR protein is required for immunomodulation by vardenafil.

Conclusion: Taken together, our results indicate that macrophages display a pro-inflammatory profile and play a critical role in inflammatory responses in CF. Moreover, the immunomodulatory effect of vardenafil, which could thus be beneficial in CF pharmacotherapy, requires CFTR expression.

33 Procalcitonin and C reactive protein levels in hospitalised patients receiving intravenous antibiotics

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Objectives: Procalcitonin (PCT), a precursor of calcitonin is selectively raised in bacterial infections. The aim of this pilot study was to investigate the role of PCT as a surrogate marker of infection in adults with CF and to discriminate between infection and inflammation in patients with elevated C reactive protein (CRP).

Methods: Adults with CF receiving hospital intravenous antibiotics for a pulmonary exacerbation were recruited. PCT levels were prospectively measured in patients with elevated C reactive protein (CRP).

Results: To date 11 subjects, median (range) age, 32 (21–51) yrs have been investigated. CRP and Procalcitonin levels were 185 (69–283) mg/L and 0.3 (<0.01–49.8) ug/L respectively. In five cases, PCT levels were \leq 0.2 ug/L despite significant elevation in CRP. In two cases PCT levels were \leq 0.1 ug/L and a clinical diagnosis of viral illness or a Tazocin reaction were suspected. In both cases full resolution occurred after discontinuing antibiotics. The highest PCT levels occurred in a patient with a CRP of 381 mg/L and following severe Influenza A H3 (seasonal) infection.

Conclusion: PCT is a more specific marker of severe infection than conventional markers such as CRP. Preliminary data suggests that PCT may be useful in discriminating between severe pulmonary and endobronchial infection as well as noninfectious inflammation. This study is ongoing.

34 Phagocyte collaboration for the control of *Pseudomonas aeruginosa*

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Pseudomonas aeruginosa (PA) is an opportunistic pathogen that causes infections in patients with compromised immunity. Several previous studies are animal based, but we have established a human neutrophil/macrophage PA infection model to investigate phagocyte collaboration in PA control.

Objectives:

- to investigate the ability of human macrophage-neutrophil co-cultures to control *Pseudomonas* growth, promote inflammation and cause tissue damage, and
- to determine how the presence of pro-inflammatory cytokines such as IFN- γ and IL-17A affect the outcome of the infection of macrophage-neutrophils co-cultures with PA.

Methods: A robust procedure has been established where, neutrophils or macrophages or both are infected with PA with or without cytokines under opsonic and or non-opsonic conditions, and the samples analysed for bacterial colony forming units (CFU) or Flow cytometry.

Results: The findings so far indicate:

- macrophages have a minor effect on the control of PA but coordinate the immune response to PA infection;
- phagocytosis of PA by neutrophils under non-opsonic conditions depends on cell density;
- as expected, serum enhances neutrophil-mediated killing of PA;
- IL-17A enhances neutrophil-mediated killing of PA under opsonic conditions and
- neutrophils synergise with macrophages in clearing PA infection.

Conclusions:

- Macrophages coordinate the immune response to PA by producing a number of cytokines.
- Neutrophil control of PA infection is enhanced by human complement.
- The cytokine IL-17A enhances the ability of human neutrophils to control PA infection.
- Neutrophils and macrophages collaborate in clearing PA infections.

35 Deficiency of the Planar Cell Polarity protein CELSR3 in CF bronchial epithelial cells increases susceptibility to epithelial–mesenchymal transition

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Objectives: Epithelial barrier function is impaired in CF airways as an indirect consequence of F508del-CFTR trafficking defect. The role of apico-basal polarity is crucial in the process but little is known about the influence of Planar Cell Polarity (PCP) which coordinates alignment of cell polarity across the tissue plane.

Methods: Expression of several PCP proteins including atypical cadherin CELSR3 (Cadherin EGF LAG seven-pass G-type receptor 3) were quantified in normal and CF primary human bronchial epithelial (HBE) cells and HBE cell lines. To characterize the role of CELSR3 in epithelial polarity, CELSR3, epithelial and mesenchymal markers expression were quantified after inducing epithelial–mesenchymal transition (EMT) in HBEs monolayers with TGF β or cisplatin. To determine if CELSR3, suspected to undergo homophilic interactions to establish cell–cell interactions, is a key determinant of epithelial barrier function, transepithelial electric resistance (TEER) was measured after silencing CELSR3 expression in 16HBEs.

Results: HBEs expressed several PCP proteins, including CELSR3 for which expression was downregulated in CF-HBEs compared to non-CF cells. Both TGF β and cisplatin induced concomitant loss of CELSR3 and epithelial marker ZO-1 and gain of mesenchymal (i.e. profibrotic) markers vimentin and fibronectin. CELSR3 siRNA in 16HBE cells enhanced TGF β -induced EMT. However, CELSR3 siRNA did not modulate TEER, indicating that CELSR3 is an epithelial marker but not a direct determinant of intercellular junction tightness.

Conclusion: CELSR3 is an epithelial marker down-regulated in CF-HBEs. Loss of CELSR3 function may confer a profibrotic phenotype to CF-HBEs cells.